Activation of unfolded protein response and autophagy during HCV infection modulates innate immune response

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Abstract. Autophagy, a process for catabolizing cytoplasmic components, has been implicated in the modulation of interactions between RNA viruses and their host. However, the mechanism underlying the functional role of autophagy in the viral life cycle still remains unclear. Hepatitis C virus (HCV) is a single-stranded, positive-sense, membrane-enveloped RNA virus that can cause chronic liver disease. Here we report that HCV induces the unfolded protein response (UPR), which in turn activates the autophagic pathway to promote HCV RNA replication in human hepatoma cells. Further analysis revealed that the entire autophagic process through to complete autolysosome maturation was required to promote HCV RNA replication and that it did so by suppressing innate antiviral immunity. Gene silencing or activation of the UPR-autophagy pathway activated or repressed, respectively, IFN-β activation mediated by an HCV-derived pathogen-associated molecular pattern (PAMP). Similar results were achieved with a PAMP derived from Dengue virus (DEV), indicating that HCV and DEV may both exploit the UPR-autophagy pathway to escape the innate immune response. Taken together, these results not only define the physiological significance of HCV-induced autophagy, but also shed light on the knowledge of host cellular responses upon HCV infection as well as on exploration of therapeutic targets for controlling HCV infection.

The treatment of chronic hepatitis C, based on the combination of Pegylated Interferon plus ribavirin, is only associated to approximately 60% sustained virological response. The development of new treatments is based on either targeting the virus or the host [1]. Therefore, it is highly important to understand the molecular interactions of the virus with the cell.

Autophagy regulates degradation of cytoplasmic components such as proteins and organelles. This process contrasts with the ubiquitin proteasome degradation pathway which specifically recognizes ubiquitinated proteins for proteasomal degradation. Autophagy is a multistep mechanism initiated by the formation and the assembly of an isolated membrane, called the phagophore, around a portion of cytoplasm. The membrane extends to form a closed autophagosome that then fuses with a lysosome to form the autolysosome (or autophagolysosome) [2]. The inner membrane and cytoplasmic components are captured and degraded by lysosomal activities. More than 31 autophagy related genes (ATG), whose products regulate the different steps of autophagy, have been identified. The most typical trigger of autophagy is nutrient starvation. Lack of any type of essential nutrient such as amino acid, nitrogen or carbon can induce autophagy. Moreover, autophagy regulates the constitutive turnover of cytosolic organelles critical for cell survival [2].

The unfolded protein response (UPR) detects cellular misfolded and unfolded proteins and targets its degradation by the autophagy machinery. Newly synthesized and secretory proteins are correctly folded and assembled in the endoplasmic reticulum (ER). During a cellular stress, the ER loses its capacity to correct protein folding, leading to the accumulation of unfolded proteins. In response to ER stress, the UPR targets the degradation of the proteins accumulated in the ER and activates the transcription of genes encoding chaperones, lectins, and calcium pump activities that serve to increase the ER’s protein folding capacity [3].

Many studies demonstrated that autophagy is activated during viral and bacterial infections. Autophagy has an antimicrobial role by restricting intracellular microbial replication and/or by maintaining the viability of infected cells. Many viruses have evolved to counteract autophagy antiviral activity [4]. Indeed, mouse hepatitis virus, poliovirus, dengue virus, and coxsackie virus use elements of the autophagy machinery as membrane scaffold to allow their own replication [4]. Different studies described a critical role for autophagy during HCV infection [5–11]. Using Huh 7.5 HCV infected cells, Sir et al. reported that HCV induces autophagic vacuoles and an incomplete autophagy to allow its replication [7]. Interestingly, using Huh 7 HCV infected cells, Dreux et al. showed that autophagy is only required for initiation of the HCV incoming replication and not to maintain replication of an established infection. Indeed, in Huh7 harboring...
the replicons H77, Con1, and JFH1, down regulation of Atg proteins does not modify viral proteins expression [6]. Interestingly, another study showed that knocking down Atg7 drastically reduces production of HCV particles while it does not modify the expression of both viral RNA and proteins in infected cells [9]. Furthermore, a recent report showed that the knock-down of either autophagy related protein 1 (BCN1) or Atg7 inhibited HCV replication in immortalized cells (IHHs) [11].

Moreover, the Atg5–Atg12 conjugate, a major component of the autophagic machinery, has been shown to directly associate with RIG-I, MDA5, and MAVS via their Cards domain [12]. These interactions result in the inhibition of IFNβ production. HCV counteracts the antiviral activity of IFN signaling. Indeed, the core protein activates SOCS3, resulting in the inhibition of Jak/STAT signaling, and the viral protease NS3/4A induces MAVS proteolytic cleavage to limit the production of IFNβ [13].

The recent study by Ke and Chen [14] highlights the importance of the complete UPR autophagy machinery during HCV infection. For the first time, the authors elegantly described that autophagy positively regulates HCV replication through its inhibitory activity on innate immunity. Ke et al. demonstrated that HCV induces the autophagy machinery through activation of the UPR machinery. Complete autophagy is needed for HCV RNA replication. In Huh7 HCVcc infected cells, knocking down members of the UPR-autophagy machinery (Atg5, CHOP, Ire1a, ATF6, and Perk) induced reduction of intracellular HCV RNA levels. Moreover, the addition of drugs inhibiting lysosome acidification, such as chloroquine, reduced the expression of NPSA protein in Huh7 HCVcc infected cells. Interestingly, the activation of IFNβ promoter by RIG-I was increased in Huh7 HCVcc infected cells with incomplete autophagy (either knocked down for Atg5 and CHOP).

Pathogen-associated molecular pattern (PAMPs) are small molecules of RNA produced from viral genome during infection. PAMPs recognition activates RIG-I and stimulates the cellular cascade leading to the production of IFNβ [10]. The authors used viral PAMPs to assess whether autophagy can modulate IFNβ stimulation through regulation of PAMPs signaling. HCVcc infected cells stimulated by HCV PAMPs showed a reduction in IFNβ production. The authors described that induction of IFNβ through HCV PAMPs was also occurring in uninfected cells and that autophagy regulated PAMPs mediated IFNβ production in both non-infected and HCV infected cells. Altogether, these results showed that autophagy negatively regulates IFNβ through viral PAMPs stimulation, and thus promotes HCV replication [14] (Fig. 1).

Several studies reported conflicting results about the importance of autophagy during HCV infection. However, these variations may be due to different experimental procedures. The use of Huh 7.5 cells, for example, might modify the observation on IFNβ activation. Indeed, whereas Huh 7.5 cells are very permissive to HCV infection, these cells harbor a defective mutation in the RIG-I gene locus which may alter IFNβ signaling pathway. Moreover, using IHHs, Shrivastava et al. demonstrated as well that autophagy regulates immune response in HCV infected hepatocytes [11]. Infection at high multiplicity of infection (MOI) may likely activate autophagy to limit the cytopathic effect of such infection. Interestingly, Ke et al. demonstrated that even at a low MOI (0.01) autophagy is activated upon HCV infection. Moreover, the authors demonstrated that HCV-induced autophagy coordinates with the infection stage.

Interestingly, Cheng et al. showed that treatment with a protease inhibitor (BILN2061) does not restore ds-RNA-induced IFNβ promoter activity in HCV infected cells [15]. These results suggest the existence of another signaling, NS3/4A independent, that may modulate IFN pathway. Ke et al. suggested that activation of autophagy might explain these observations. Moreover, Dreux et al. reported that autophagy is required only for replication of incoming viruses and not in an established infection [6]. At the beginning of the infection whereas NS3/4A is not present at a
sufficient level, autophagy might be activated to limit IFNβ production. Conversely, when the infection is established, the expression of NS3/4A might be high enough to regulate IFNβ production and it might explain why Dreux et al. did not report an effect of autophagy in an established infection.

It is likely that HCV is using autophagy to promote its replication because autophagy primarily has an antiviral activity. HCV might encode an activity able to regulate the UPR-autophagy machinery. Ke et al. results suggest that HCV RNA replication is the mechanism that induces UPR and autophagy.

In vivo, few studies investigated the role of autophagy during HCV infection. We previously described an activation of the ER stress and UPR sensors in liver biopsies of HCV infected patients [16]. Whereas ER stress related genes showed higher expression in HCV patients, UPR related genes were not significantly up-regulated [16]. In this study, the autophagy activity was not investigated in vivo. The absence of UPR genes up-regulation is not necessarily associated with normal activity of UPR and autophagy. Autophagy activation in vivo might be induced by hepatocytes ER stress. Ke et al. knocked down either the UPR or autophagy components [14]. Since, upstream, ER stress is able to activate UPR, it would be interesting to investigate if knocking down a component of ER stress would have the same effect on IFNβ activation than knocking down UPR and autophagy components.

A recent study performed in our group showed an increased autophagic response in patients with chronic hepatitis C in comparison with patients with no or mild fibrosis, NASH, and chronic hepatitis B [17]. Moreover, autophagic vesicles accumulate in hepatocytes independently of the HCV genotype. Although electron microscopy showed a strong accumulation of autophagosome vesicles, mature lysosomes do not increase in hepatocytes. These observations are in accordance with previous in vitro studies reporting an incomplete autophagy in HCV infected cells [7].

Interestingly, two independent studies reported that IFNβ production is increased in HCV infected cells harboring incomplete autophagy [11,14]. Thus, in response to IFNβ stimulation, interferon stimulated genes (ISGs) might be up-regulated in infected and neighbor cells. This is a very interesting finding since anti HCV therapy is based on the combination of PEG-IFNα plus ribavirin. Moreover, several reports showed that a high level of expression of ISGs before the treatment is associated with non response [18–20]. These observations raise the hypothesis that autophagy might be down-regulated in non responders, resulting in an increased expression of ISGs.

Cyclophilins are molecular chaperones encoding a peptidyl-prolyl cis–trans isomerase activity. As chaperons, cyclophilins target misfolded proteins to the UPR autophagy machinery. Interestingly, it has been reported that the addition of an inhibitor of cyclophilins, cyclosporin A, reduced the activity of autophagy induced by nutrient starvation [21]. Moreover, several reports demonstrated that cyclophilins are required for HCV replication [9,22–24]. In vitro, cyclophilin A shows a potent antiviral effect against HCV replication. Debio 025 is an inhibitor of Cyp that derives from cyclosporine A and does not display calcineurin inhibition. A phase II study in treatment-naïve patients with chronic hepatitis C confirmed that Debio 025 has a potent activity [25]. Since cyclosporine A reduces autophagy in vitro, Debio 025 might also restrict autophagy activity in patients. Ke et al. reported that inhibition of autophagy increases the production of IFNβ [14]. Altogether, these results suggest that cyclophilin inhibitors may restrict HCV replication and innate immune response through inhibition of autophagy.

Interestingly, Ke et al. described a new role of autophagy in the regulation of innate immunity during HCV infection [14]. HCV may encode an NS3/4A independent activity that triggers autophagy to limit the production of IFNβ.

Cyclophilin inhibitors might act as a potent anti-autophagy agent and limit both inhibition of innate antiviral response and HCV replication.

Conflict of interest

Tarik Asselah is a speaker and investigator for BMS, Boehringer Ingelheim, Tibotec, Janssen, Cilag, Gilead, Roche, Merck, and Schering-Plough.

References


